

(e) *Procedure.* Use the equipment, mobile phase, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(1) of this section.

(1) *System suitability test.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by three replicate injections of 10 microliters each of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the tailing factor, efficiency of the column, coefficient of variation, and capacity factor as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the following minimum performance requirements have been met:

(i) *Tailing factor.* The tailing factor is satisfactory if it is not more than 1.2;

(ii) *Efficiency of the column.* The efficiency of the column is satisfactory if it is greater than 1,900 theoretical plates;

(iii) *Coefficient of variation.* The coefficient of variation of at least three replicate injections is satisfactory if it is not more than 1.5 percent; and

(iv) *Capacity factor.* The capacity factor is satisfactory if it is not less than 1.8 and not more than 5.

If the minimum performance requirements are not met, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the ceforanide content as directed in the

individual monograph for the drug being tested.

[49 FR 25846, June 25, 1984; 49 FR 34347, Aug. 30, 1984]

§ 436.349 High-pressure liquid chromatographic assay for L-lysine in ceforanide for injection.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A suitable pump capable of reproducibly delivering a liquid to a pressure of 5,000 pounds per square inch;

(2) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(3) A suitable recorder;

(4) A suitable integrator; and

(5) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) 2,4-Dinitrofluorobenzene solution. Weigh accurately approximately 760 milligrams of 2,4-dinitrofluorobenzene into a 50-milliliter volumetric flask. Dissolve and dilute to volume with absolute ethyl alcohol.

(2) Tris (hydroxymethyl) aminomethane (THAM) solution. Weigh accurately approximately 1.44 grams of THAM into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water.

(c) *Mobile phase.* Mix methanol and water (62:38), and adjust to pH 3.0 with glacial acetic acid.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the standard that is at least 50 percent of scale with a typical chart speed of 0.2 inch per minute.

(e) *Preparation of standard and sample solutions*—(1) *Preparation of standard solution.* Weigh accurately approximately 36 milligrams of L-lysine used as the standard into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer 2.0 milliliters of the L-lysine solution into a 10-milliliter volumetric flask,

add 2.0 milliliters of THAM solution and 3.0 milliliters of 2,4-dinitrofluorobenzene solution. Cap tightly and mix well. Place the flask in a 50° C water bath for 30 minutes. Remove from water bath, allow the flask to cool to room temperature, and dilute to volume with methanol. Mix well.

(2) *Preparation of sample solution.* Weigh accurately approximately 150 milligrams of the sample, ceforanide for injection, into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer 2.0 milliliters of the sample solution into a 10-milliliter volumetric flask, add 2.0 milliliters of THAM solution and 3.0 milliliters of 2,4-dinitrofluorobenzene solution. Cap tightly and mix well. Place the flask in a 50° C water bath for 30 minutes. Remove from water bath, allow the flask to cool to room temperature, and dilute to volume with methanol. Mix well.

(f) *Procedure.* Use the equipment, reagents, mobile phase, operating conditions, and standard and sample solutions described in paragraphs (a), (b), (c), (d), and (e) of this section, and proceed as directed in paragraph (f)(1) of this section.

(1) *System suitability test.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by three replicate injections of 20 microliters each of the standard solution. Allow an elution time sufficient to obtain satisfactory separation of the expected components after each injection. Record the peak responses and calculate the resolution factor, tailing factor, efficiency of the column, coefficient of variation, and capacity factor as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (f)(2) of this section if the following minimum performance requirements have been met:

(i) *Resolution factor.* The resolution factor between the peak for derivatized *L*-lysine and from the peak for the dinitrofluorobenzene derivatizing reagent is satisfactory if it is not less than 4.5;

(ii) *Tailing factor.* The tailing factor is satisfactory if it is not more than 1.3;

(iii) *Efficiency of the column.* The efficiency of the column is satisfactory if it is greater than 1,500 theoretical plates;

(iv) *Coefficient of variation.* The coefficient of variation of at least three replicate injections is satisfactory if it is not more than 1.5 percent; and

(v) *Capacity factor.* The capacity factor is satisfactory if it is not less than 4 and not more than 6.

If the minimum performance requirements are not met, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (f)(2) of this section.

(2) *Determination of the chromatogram.* Inject 20 microliters of the standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the standard solution is completed, inject 20 microliters of the sample solution into the chromatograph and repeat the procedure described for the standard solution.

(g) *Calculations.* Calculate the percent of *L*-lysine per milligram of ceforanide for injection as follows:

$$\text{Percent of } L\text{-lysine} = \frac{A_u \times P_s}{A_s \times C_u} \times 10$$

A_u = Area of the *L*-lysine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the *L*-lysine peak in the chromatogram of the *L*-lysine standard;

P_s = *L*-lysine content in the *L*-lysine standard solution in micrograms per milliliter; and

C_u = Milligrams of sample per milliliter of sample solution.

[49 FR 25846, June 25, 1984; 49 FR 34347, Aug. 30, 1984; 49 FR 40006, Oct. 12, 1984]

§ 436.350 High-performance liquid chromatographic assay for cefonicid.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with: